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Chapter 103

Taxonomy

EMILIO WEISS

Section 9 of *Bergey's Manual of Systematic Bacteriology* (6) describes in detail the orders *Rickettsiales* and *Chlamydiales*. The *Rickettsiales*, even though separated from the endosymbionts in this edition of *Bergey's Manual*, represent a complex taxon, which includes the families *Rickettsiaceae*, *Bartonellaceae*, and *Anaplasmataceae*. The *Chlamydiales*, on the other hand, include a single family and a single genus, possibly underclassified. Numerous observations, isolations of new species, and phylogenetic analyses made during the past decade and for the most part after the publication of *Bergey's Manual* suggest that we should take another look at the classification of the family *Rickettsiaceae* and the genus *Chlamydia*.

Table 1 lists the species in the family *Rickettsiaceae* and in the genus *Chlamydia*, including species that have recently been added, and summarizes the information thus far obtained through comparisons of 16S rRNA sequences (20).

Among the changes that have occurred during the past decade, human infection in Europe with the spotted fever group rickettsia *Rickettsia conorii* was found to occur more frequently and to produce a more severe disease than in the past (10, 11). To the large number of rickettsial species in the spotted fever group, some established pathogens and others most likely commensals in the invertebrate host, two have been added, the pathogen *R. japonica* (14) and the commensal *R. helvetica* (8). The commensal *R. bellii* was isolated and described as a rickettsia that is possibly not a member of the spotted fever group (9).

The most extensive additions to our information are possibly those that involve the genus *Ehrlichia*. Ristic et al. (12) demonstrated that there is a small but highly significant degree of serologic relationship between the dog pathogen *Ehrlichia canis* and the human pathogen *Rickettsia sennetsu*. This finding was quickly followed by the observations that the two microorganisms strongly resemble each other in morphology and mode of development (3). As a result the sennetsu agent was reclassified in *Bergey's Manual* as *Ehrlichia sennetsu*. A few years later the etiologic agent of Potomac horse fever (equine monocytic ehrlichiosis) was isolated and found to be distantly related to *E. canis* and more closely related to *E. sennetsu* (4, 5). The evolutionary history of *E. sennetsu* and *E. risticii*, two microorganisms that are biologically similar but that differ widely in geographic distribution and pathogenesis for vertebrate hosts, well deserves to be investigated. A possible link between the two microorganisms is suggested by the recent discovery (discussed in chapter 106) that *E. canis*, or a closely related microorganism, is also a human pathogen.

Also worth mentioning is the similarity between another dog pathogen and a potential human pathogen. *Neorickettsia helminthoeca* is a dog pathogen that occurs in the U.S. West Coast states. Dogs acquire the disease by eating raw salmon infested with the metacercariae of a trematode, which in turn is infected with *N. helminthoeca* (6). Fukuda et al. (1) presented evidence that a disease involving a similar cycle may occur in Japan among people who eat improperly cooked fish. The fish, the grey mullet, is also often infested with a trematode that carries a bacterium similar to *N. helminthoeca*.

To the genus *Chlamydia*, the important human pathogen *C. pneumoniae* has been added (2).

The study of the phylogeny of the *Rickettsiaceae* and of chlamydiae conducted thus far has produced some results that confirm observations made on phenotypic similarity among certain species, while others contrast sharply with our present taxonomic concepts. For example, as expected, *Rickettsia prowazekii*, *R. typhi*, and *R. rickettsii* were shown to have very similar rRNA sequences (differences of <2%, smaller than the typical difference between the genera *Escherichia* and *Salmonella*) (15). These three rickettsial species belong to the alpha subdivision of the purple bacteria (20), recently named *Proteobacteria* (13). In this subdivision, the rickettsial species are specifically, although distantly, related to *Ehrlichia risticii* (15). This came as a surprise, since rickettsiae and the monocytic ehrlichiae differ in morphology and intracellular location, cytoplasm in the case of rickettsiae and phagosome in ehrlichiae (6). On the other hand, it has recently been shown that ehrlichiae and rickettsiae resemble each other in in vitro substrate utilization and gain in ATP content from this activity (18, 19). The rickettsiae are not specifically related to *Rochalimaea quintana*. This microorganism is also a member of the alpha subdivision of *Proteobacteria*, but belongs to subgroup 2 and is specifically related to the plant-associated agrobacteria and rhizobacteria (17). This is also unexpected since *R. prowazekii* and *R. quintana* have historically been transmitted by the same arthropod vector, the body louse, and the two DNAs hybridize with each other to the extent of 25 to 33% (7).

Not surprising, because of marked differences in phenotypic characteristics, was the finding that *Coxiella burnetii* is unrelated to the three species of rickettsia. It belongs in the gamma subdivision of the *Proteobacteria* and in this group it shows a rather distant but specific relationship to the genus *Legionella*. The tick commensal, *Wolbachia persica*, was also shown to belong in the gamma subdivision and to be peripherally related to the *Coxiella-Legionella* cluster (15).

Most astonishing was the finding that chlamydiae have no close relatives among the eubacteria (16). They represent a hitherto unrecognized major eubacterial group, peripherally related only to the planctomyces. These

TABLE 1. List of species in the family *Rickettsiaceae* and genus *Chlamydia* and summary of present knowledge of their phylogeny based on 16S sRNA sequences[a]

Genus	Group	Species	Proteobacterial subdivision[b]	Related genus[b]
Rickettsia	Typhus	*R. prowazekii*,[c] *R. typhi*[c]	Alpha	*Ehrlichia*
		R. canada	Unknown	Unknown
	Spotted fever	*R. rickettsii*[c]	Alpha	*Ehrlichia*
		R. conorii,[c] *R. sibirica*,[c] *R. australis*[c]	As above	As above
		R. akari,[c] *R. japonica*[c]	As above	As above
		R. montana, *R. parkeri*, *R. rhipicephali*	Unknown	Unknown
		R. helvetica	Unknown	Unknown
	Uncertain	*R. bellii*	Unknown	Unknown
	Scrub typhus	*R. tsutsugamushi*[c]	Unknown	Unknown
Rochalimaea		*R. quintana*[c]	Alpha, subgroup 2	Agrobacteria, rhizobacteria
		R. vinsonii	Unknown	Unknown
Ehrlichia	Monocytic	*E. risticii*[d]	Alpha	*Rickettsia*
		E. canis,[c,d] *E. sennetsu*[c]	As above	As above
	Granulocytic	*E. equi*,[d] *E. phagocytophila*[d]	Unknown	Unknown
Cowdria		*C. ruminantium*[d]	Unknown	Unknown
Neorickettsia		*N. helminthoeca*[d]	Unknown	Unknown
Coxiella		*C. burnetii*[c,d]	Gamma	*Legionella*
Wolbachia		*W. persica*	Gamma	*Coxiella*, *Legionella*
		W. pipientis, *W. melophagi*	Unknown	Unknown
Rickettsiella[e]		*R. popilliae*, *R. grylli*, *R. chironomi*	Unknown	Unknown
Chlamydia		*C. trachomatis*,[c] *C. psittaci*[c,d]	None (not a proteobacterium)	*Planctomyces*
		C. pneumoniae[c]	As above	As above

[a] The *Rickettsiales* families *Bartonellaceae* and *Anaplasmataceae* are not included. Not all of the species listed are discussed in subsequent chapters.

[b] "As above" below a proteobacterial subdivision or genus designation means that it is reasonable to assume that the designation above is applicable. Such an inference cannot be made for some of the other species of the same genus.

[c] Established human pathogen.

[d] Animal pathogen.

[e] Pathogenic for invertebrate host.

two groups of bacteria have very little in common phenotypically, except that the cell wall in both cases contains no peptidoglycan.

Much remains to be done in the field of phylogeny of the rickettsiae. If this work is extended to other pathogens of the spotted fever group, such as *R. conorii* or *R. sibirica*, few surprises are expected, because of the solid base of phenotypic information, which suggests that these rickettsiae are closely related. However, *R. tsutsugamushi* is sufficiently different from the other rickettsiae to deserve separate phylogenetic analysis. Despite the large body of information that we have on this species, in the absence of such a study we cannot venture to say that it is a proteobacterium.

The place of taxonomy in a manual of clinical microbiology is not clear. When phylogenetic information reinforces what we have learned by the study of phenotypic characteristics, it encourages us to proceed as we have. When a conflict develops between genotypic and phenotypic information, for the immediate purpose of isolation and identification of pathogens, it is preferable to pay greater attention to the phenotypic characteristics. However, genotypic information cannot be ignored. A conflict may suggest that important biologic or ecologic characteristics of the microorganisms involved are yet to be uncovered.

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Taxonomy and the study of the bacterial genome have recently acquired new significance as the polymerase chain reaction (PCR) is being developed for the rapid identification of infectious agents (K. E. Hechemy, D. Paretsky, D. H.Walker, and L. P. Mallavia, ed., Ann. N.Y. Acad. Sci. **590**:1–586, 1990). For example, although the scrub typhus group of rickettsiae is still represented by a single species, *R. tsutsugamushi*, there is considerable variation in antigenic properties among the various strains. This variation is not necessarily accompanied by marked differences in virulence for humans. Heterogeneity has been attributed to variability of certain surface components that occur in a background of strong homology (E. V. Oaks, R. M. Rice, D. J. Kelly, and C. K. Stover, Infect. Immun. **57**: 3116–3122, 1989). It is therefore important, for the purpose of the PCR test, to select a region on the genome that is relatively stable and recognizes most, if not all, strains. In the case of the typhus and spotted fever group rickettsiae, there is sufficient similarity in their genomes for the selection of a region that detects several species, such as *R. prowazekii*, *R. typhi*, *R. rickettsii*, and *R. conorii*. If the test is positive, the identity of the infectious agent can, in most cases, be inferred on the basis

of geography and other epidemiological considerations. Although more specific PCR tests can be applied, even if the species is not recognized in the initial test, treatment need not be delayed, since all of the above rickettsial species are susceptible to tetracycline or doxycycline. It has been recognized, however, that rickettsiae share antigens with several other bacterial species, including in some cases *Legionella* species. A genome that recognizes *Legionella* species should not be included in any test for rickettsiae, since the antibiotic of choice for *Legionella* species is erythromycin, which is not effective against rickettsiae (Hechemy et al., Ann. N.Y. Acad. Sci. **590**: 1–586, 1990).

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